



Part #7

033166-017

Marked-Up Copy of Substitute Specification Filed July 9, 2003

BOTANICAL / COMMERCIAL CLASSIFICATION

Cymbopogon commutatus / Cymbopogon Plant

VARIETAL DENOMINATION

cv. 'RLJCC1'

RECEIVED

JUL 16 2003

TECH CENTER 1600/2900

**FIELD OF THE INVENTION**

The present invention relates to ~~development of~~ a drought tolerant ~~strain~~ variety of ~~Cymbopogon~~ Cymbopogon commutatus plant that is rich in geraniol and geranyl acetate ~~designated as Cymbopogon RLJCC1~~ named 'RLJCC1'.

**BACKGROUND OF THE INVENTION**

~~The~~ Indian sub-continent is has a rich in genetic diversity in aromatic plants. The aromatic grasses ~~viz., Cymbopogon~~ such as Cymbopogon and ~~Vetiveria zizanoides~~ Vetiveria zizanoides have been used by man from ancient times both in for medicine and perfumery. ~~Genus Cymbopogon~~ The genus Cymbopogon, belonging to family ~~Poaceae~~, are Poaceae, is a rich ~~sources~~ source of aroma chemicals, especially terpenoids. These chemical compounds are present in varying concentrations in ~~the species, varieties, ecotypes or chemotypes of~~ Cymbopogon Cymbopogon have great scope for utilization and are used widely in

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

perfumery, and in the flavor and pharmaceutical ~~industry industries~~. There are ~~upto~~ up to 60 species of ~~Cymbopogon~~ Cymbopogon native to the tropical and sub-tropical regions of Africa and Asia. ~~See, Corrigan, D. (1992). In: "Adverse Effects on Herbal Drugs", Vol. I, Springer verlag, Berlin, pages 115-123. Out of 27 species available in India, mainly C. flexuosus, C. winterianus~~ C. flexuosus, C. winterianus and C. martinii C. martinii, var. ~~motia~~ 'Motia' have been exploited for commercial cultivation as a source of citral, citronellal and geraniol respectively. ~~Cymbopogon Commutatus (Steud.) Stapf~~ Cymbopogon commutatus is surviving in the sub-tropical environment of the Jammu ~~district~~ District near R. S. Pura Tehsil, India. ~~The~~ A massive collection of this plant was made in India during ~~monsoon season~~, 1994.

The occurrence of ~~Cymbopogon Commutatus~~ Cymbopogon commutatus is reported ~~from~~ in Sudan Banthorpe, D.V., Duprey, R.J.H., Hassan, M., Janes, J.F. and Modawi, B.M. (1976). ~~Planta Medica~~ Planta Medica 29:10-19. Such plant also is present in Pakistan, Somalia, Tanzania, Iraq, and Northern India. ~~See, Nasir, E. and Ali, S.I. (1982). "Flora of Pakistan - Poaceae", No. 143. University of Karachi, Karachi, Pakistan.~~

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

India's share in land resources of the world is only 2% on which 18% of the world's population and 15% of the world's livestock survive. The geographical matrix of India based on the reported area of 305.01 million hectares is broadly grouped into three sectors -- the agriculture sector (59.27%), the ecological sector (33.56%), and the non-agricultural sector (7.17%). See, Prasad, R.N. and Biswas, P.P. (2000), "Land resource in sustainable agricultural development –issues and strategies Indian Farming", 49(11): Pages 9-13. Dryland agriculture in India is now practiced on 100 million hectares or 70% of the total arable land of 143.8 million ha. Crop production on these lands is dependent entirely on natural precipitation. This information suggests that there is need in India and elsewhere to develop an improved drought tolerant variety of ~~C. commutatus~~ C. commutatus.

Present annual demand of geraniol in India stands at about 100 tonnes which is likely to increase during the coming years. In India, the current production is only 50 tonnes/year. The geraniol containing oil can be used for imparting ~~rosaceous~~ an aroma to the wide range of perfumery products ~~from~~ perfumery.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

The Polymerase Chain Reaction (PCR) technique has found ~~a number of~~ applications wide use in the molecular biology. Recent advances in PCR have made this technique one of the most powerful tools for a wide spectrum of molecular ~~analysis analyses~~, such as genome mapping. See, Benito C., Figueiras, A.M., Zaragoza, C., Gallego, F.J., and De la Pena, A., (1993)-, Plant Mol. Biol., 21:181-183-; molecular evolution, Brown, P.T.H., Lange, F.D., Kranz, E, and Lorz, ~~Hhhh~~ H., (1993)-, Mol. Gen. Genet., 237: 311-317-; gene tagging-; molecular taxonomy-; ~~diagnosis~~ diagnosis of genetic diseases, and forensic sciences, Erlich, H.A.D., Gelfand, and J.J.Sninsky- (1991)-, Science 252: 1643-1651. Randomly Amplified Polymorphic DNA profiling (RAPD) is one of the PCR techniques which is an amplification-based nucleic acid scanning technique driven by synthetic oligodeoxynucleotide primers of arbitrary sequence producing characteristic DNA fingerprints capable of detecting sequence polymorphism in anonymous nucleic acid templates. In this technique the amplification of genomic DNA using random short primers results in multiple amplification products representing amplicons randomly distributed throughout a genome which can be resolved by agarose gel electrophoresis and visualized by ethidium bromide staining.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

The polymorphism obtained using RAPD ~~result~~ results from point mutations, insertions, deletions, and inversions occurring in the respective genomes ~~in due course of over~~ time. ~~RAPDs~~ These are usually dominant markers that are inherited in simple Mendelian fashion. No references ~~are~~, however, ~~available~~ are known to exist on the RAPD analysis of ~~Cymbopogons~~ Cymbopogons. ~~However the~~ The methodology used by D.Godwin, N.Sangduen, R.Kunanuvatchaidach, G.Piprridis, and ~~S.W.adkins~~. S.W. Adkins (1996)-, Plant Cell Reports, 16: 320-324; Taku Ohmori, Minoru Murata and Fusao Motoyoshi- (1995)-, Jpn. J. Genet. 70; 179-184; F.N.Wachira, R.Waugh, C.A.Hackett, and W.Powell-(1994)-, Genome 38: 201-210, ~~have~~ has been used for the present studies to further confirm the distinctiveness of the new variety of the present invention.

A ~~very well established~~ well-established method of mass selection for developing a ~~better strain~~ an improved variety from the wild collection of ~~Cymbopogon Commutatus~~ Cymbopogon commutatus was ~~adopted~~ initiated during 1994 and individual plant progenies were raised vegetatively by slips in a cultivated area tended by man.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

Similar ~~looking~~ appearing uniform progenies having the desired phenotypic characters, ~~e.g. e.g.,~~ an improved tiller plant character, high rate of tillerization, fresh herbage, essential oil content (%), and oil quality indices (ratio of geraniol/geranyl acetate to citral) were bulked for seed formation.

Seed raised progenies exhibited phenotypic variations, ~~and a~~ A single plant of desired characters ultimately was selected ~~and designated as RLJCC1~~ and was further multiplied vegetatively. This plant of the present invention was designated 'RLJCC1'. ~~The studies~~ Studies and selection were continued from 1995 to 1999 for ~~further~~ the evaluation of essential oil quality stability ~~and population~~ adaptability (i.e., geraniol and geranyl acetate) and drought tolerance.

~~The best plants were identified based on their superiority and outstanding drought resistibility. These were again screened out to produce a new population where after further cycles of selection were carried out based on drought resistance and chemical constituents, such as geraniol and geranyl acetate.~~

**~~OBJECTS OF THE INVENTION~~**

The primary object of the present invention is was to develop new ~~Cymbopogon strain~~ *Cymbopogon commutatus* variety capable of growing in under natural drought conditions.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

Another object of the present invention is was to develop a ~~Cymbopogon~~ strain new *Cymbopogon commutatus* variety which exhibits the presence of geraniol and geranyl acetate as major chemical constituents and having a low citral content for applications in the perfumery and flavor ~~industry~~ industries.

Yet another object of the present invention is was to develop a ~~Cymbopogon strain~~ new *Cymbopogon commutatus* variety useful ~~for utilizing in~~ in the marginal as well as in waste ~~land through cultivation~~ lands for the production of geraniol and geranyl acetate as well as ocimene ~~and also to generate~~ employment of peoples of different section of societies which include farming communities, tribal communities and weaker section of the society.

**SUMMARY OF THE INVENTION**

~~This invention relates to the isolation and development of drought tolerant variety of Cymbopogon Commutatus named as RLJCC1 through mass selection technique which is potential source of geraniol and geranyl acetate of over 80% and optimal oil content (0.4-0.5%) on fresh weight basis and characterized as drought tolerant having perennial densely tufted grass and rich herbage with mass flowering pattern.~~

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

It was found that the new *Cymbopogon commutatus* variety of the present invention exhibits the following combination of characteristics:

- (a) displays a perennial vigorous growth habit.
- (b) commonly forms lighter green leaves than typically exhibited by the species.
- (c) forms an abundance of roots with superior tillering.
- (d) displays superior drought tolerance.
- (e) produces essential oil having an enhanced geraniol and geranyl acetate content, and
- (f) readily undergoes asexual reproduction by the use of slips.

**BRIEF DESCRIPTION OF THE DRAWINGS AND PHOTOGRAPHS**

The accompanying drawings and photographs provide information concerning the inherently exhibited characteristics of the new 'RLJCC1' variety. In some instances information is provided for comparative purposes with respect to the typical *Cymbopogon commutatus* plant from which the new 'RLJCC1' variety was selected and the *Cymbopogon nardus* 'RRL-CN5' variety (non-patented in the United States). The depicted plants were being grown in the field at the Jammu and Kashmir State of North India at a longitude 75°55'E, latitude 32°44'N. Such



**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

plants were reproduced by the rooting of cuttings. Slips were planted in February and flowering began in April and continued to the 5<sup>th</sup> and 6<sup>th</sup> leafing stage during May and June.

FIGURE 1 depicts a typical plant of the 'RLJCC1' variety while displaying a profusion of inflorescence-bearing shoots.

FIGURE 2 illustrates in detail typical sessile and pedicillate spikelets of the inflorescence of the 'RLJCC1' variety.

FIGURE 3 depicts a close view of the typical adventitious root system of the 'RLJCC1' variety.

FIGURE 4 depicts a typical plant of the 'RLJCC1' variety at the vegetative growth stage of development.

FIGURE 5 depicts for comparative purposes a typical *Cymbopogon commutatus* plant at the vegetative growth stage of development from which the new 'RLJCC1' variety was obtained through selection. Note the dark green coloration of the leaves.

FIGURE 6 depicts a further view of typical plant of the 'RLJCC1' variety following seed production.

FIGURE 7 depicts a mass primarily of plants of the 'RLJCC1' variety while growing in the field at the early vegetative stage of development.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

FIGURE 8 depicts the RAPD profile of the new 'RLJCC1' variety and the typical *Cymbopogon commutatus* plant (Lanes 3 and 4 respectively) using primer 22 (48 ng) and a  $\text{MgCl}_2$  concentration of 2.5 mM.

FIGURE 9 depicts the RAPD profile of the new 'RLJCC1' variety and the typical *Cymbopogon commutatus* plant (Lanes 3 and 4 respectively) using primer 27 (48 ng) and a  $\text{MgCl}_2$  concentration of 2.5 mM.

FIGURE 10 depicts the RAPD profile of the new 'RLJCC1' variety and of the 'RRL-CN5' variety of *Cymbopogon nardus* using two different primers (i.e., 27 and 29). Lane 1 shows the 'RLJCC1' variety and Lane 2 shows the 'RRL-CN5' variety using primer 27 (48 ng) and a  $\text{MgCl}_2$  concentration of 2.5 mM. Lane 3 shows the 'RLJCC1' variety and Lane 4 shows the 'RRL-CN5' variety using primer 29 (33 ng) and a  $\text{MgCl}_2$  concentration of 2.5 mM.

FIGURE 11 depicts the RAPD profile of the new 'RLJCC1' variety and of the 'RRL-CN5' variety of *Cymbopogon nardus* using primer 22 (33 ng) and a  $\text{MgCl}_2$  concentration of 2.5 mM. Lane 3 shows the 'RLJCC1' variety and Lane 4 shows the 'RRL-CN5' variety.

## Marked-Up Copy of Substitute Specification Filed July 9, 2003

~~DETAILED DESCRIPTION OF THE INVENTION~~

~~A new and distinct hybrid plant named "RLJCC1" having the following combination of characters:~~

## TAXONOMIC CHARACTERISTICS

THE CYMBOPOGON GENUS

~~Cymbopogon~~ Cymbopogon is recognized to be closely allied to ~~Andropogon~~ Andropogon and ~~Hyparrhenia~~ Hyparrhenia and it is sometimes quite difficult to ~~separate from~~ distinguish them. A useful diagnostic character is the aromatic flavor when a leaf of ~~Cymbopogon~~ Cymbopogon is chewed; with the other genera ~~are~~ being tasteless. The genus is notorious for the considerable variations within species and the weak separation between ~~them~~ species. Consequently ~~its~~ the taxonomy is still in a fluid state with differing opinions about the level at which specific rank should be accorded and with many of the species based upon indefinite characters of little practical diagnostic value.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

**~~CYMBOPOGON:~~**

Plants of the *Cymbopogon* genus commonly are tall ~~Tall~~ robust perennials, the leaf blade blades are linear, aromatic, and the ligule lingules are membranous or scarious. ~~Inflorescence~~ The inflorescence is composed of paired racemes borne on a short common peduncle and is enclosed by a ~~boat-shaped~~ boat-shaped spatheole, ~~these~~ and densely crowded into a leafy false panicle which is often very large and complex, raceme are short, with each raceme base ~~which being~~ deflexed at maturity, the lower most pair of spikelets in each raceme is homogamous ~~and resembling the pedicelled~~, and the internodes and pedicels are linear. Sessile spikelets are dorsally compressed, callus is obtuse inserted in the concave, the lower ligule is streaked with oil glands, ~~2-keeled~~ two-keeled, and the lower florets are reduced to a ~~hyaline~~ hyaline lemma with awn from the sinus. Pedicelled spikelets caryopsis are oblong.

**~~CYMBOPOGON COMMUTATUS~~**

**CYMBOPOGON COMMUTATUS (STEUD.) STAPF**

This species can be generally described as indicated hereafter.

Perennial, culms erect 15 to 150 cm high, leaf blade flat 10-50 cm long, 1-4 mm wide, dull green narrowed at the base, filiform tip, basal sheaths persistent

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

thinly pubescent, spatheoles narrowly lanceolate to narrowly elliptic, 2-2.6 cm long. Racemes 15-40 mm long, lower most pedicel swollen and ~~barrel-shaped~~ barrel-shaped internodes and ~~pedicels~~ pedicels densely ciliate along the margins glabrous to minutely puberulous on a the back. Sessile spikelets narrowly lanceolate 4-7 mm long, lower glume flattish to deeply concave on the back. Upper lemma deeply bifid, with an awn 10-20 mm long. Chromosome number  $2n=20, 40$ , Nasir, E. and Ali, S.I. (1982). Flora of Pakistan - Poaceae, No. 143. University of Karachi, Karachi, Pakistan.

**~~SELECTION STRATEGY FOR ISOLATION OF IMPROVED DROUGHT TOLERANT STRAIN OF C.COMMUTATUS~~**

~~————Cymbopogon species produce numerous intermediate forms (hybrid complexes) due to cross-pollination in nature. However, the formation of essential oil is a genetically controlled phenomenon but abiotic/biotic factors influence the oil production by stimulating the physiological processes and ultimately these changes lead to adaptation and help for the selection of a drought tolerant plant variety. Hence an improved clone RLJCC1 had been isolated by mass selection technique having same oil percentage as that of the mother plant and has better drought tolerance value than that of the parent plant. Wilde, S.A., Corey, R.F.,~~

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

~~Iyer, J.G. and Voigt, G.K. 1979. In: Soil and Plant Analysis for Tree Culture  
Oxford & IBH Publishing Co., New Delhi.~~

**~~MORPHOLOGY OF CYMBOPOGON RLJCC1~~**

~~—— It is perennial densely tufted grass, attains a height of 100 to 150 cm having  
50 cm long linear green leaf blade and 5-8 mm wide (Fig. 1, 2 & 3). It exhibited  
synchronous mass flowering pattern and triggered from autumn season onwards.~~

**~~PLANT COLOUR DESCRIPTION~~**

~~—— The color description of mother plant of *C. commutatus* and RLJCC1 are  
described as per the Methuen Handbook of colors by A. Kornerup and  
J.H. Wanscher revised by Don Parey, Third Edition, 1978, published by Erye  
Methuen, London, having arrays of 12666 color samples. The book does not  
provide rough color identification but a universal ready reference for all color  
users. Therefore, the color description has been made as under:~~

**Marked-Up Copy of Substitute Specification Filed July 9, 2003****~~MOTHER PLANT OF C.COMMUTATUS:~~**

~~———— The color of the plant is jade green exhibiting sample reference 27E5. It refers to the color of the plant, which can be found on plate 27, Column E, Row 5.~~

~~**RLJCC1:** The plant exhibited foliage green color when viewed from a distance and sample reference 30D5 i.e. plant color can be found on plate 30, Column D, Row 5.~~

**~~MORPHO-ECONOMIC CHARACTERS~~**

~~———— Data pertaining to morpho-economic characteristics of Cymbopogon Commutatus (RLJCC1) revealed that it exhibited better production of number of leaves and tillers plant<sup>-1</sup>. The rate of tillerization (RTR) is as high as 2.0, which exhibited faster growth and regrowth characteristics (Fig. 4,5,6 & 7). Essential oil yield plant<sup>-1</sup> is also optimal (Table 1).~~

~~———— Here this RAPD Analysis has been applied to develop DNA fingerprints and relatedness of accessions of Cymbopogon species developed in this laboratory (selected plant RLJCC1, its mother plant CCM and plant similar to it chemically RRL-CN5) for quality control and identification. Protocols for the isolation of~~

### Marked-Up Copy of Substitute Specification Filed July 9, 2003

genomic DNA from hybrid plant *Cymbopogon commutatus* (RLJCC1), its mother plant (CCM) and its chemically comparable plant species *C. nardus* var. *confertiflorus* (RRL-CN5) were optimized by modifying the CTAB method (Fang et al., 1992). The purity of the DNA was checked spectrophotometrically at 260/280. PCR protocols for the development of comparable & reproducible RAPD profiles in selected plant species (RLJCC1, CCM and RRL-CN5) were optimized using 40 Operon Random primers showed the comparable RAPD profiles in the above *Cymbopogon* accessions. The best profile has been developed using the random primers 22, 27 and 29 (Figs. 8 & 11).

### PLANT MATURITY AND QUALITY INDICES

Leafing and tillerization started with the rise of ambient temperature from March onwards after planting the slips in the month of February. Vegetative phase continued upto April thereafter flowering started. Floral induction continued upto 5th leafing & 6th leafing stage during May and June. Plant maturity in terms of quality indices i.e. geraniol and geranyl acetate content over 80% having optimal oil content 0.45-0.50%. Ocimene(5.35%), Linalool, (1.44%), neral(1.5%), geraniol(2.65%) are among the minor chemical constituents.



**Marked-Up Copy of Substitute Specification Filed July 9, 2003****~~PLANT STABILITY~~**

~~———— Comparative performance of essential oil quality index stability parameters of strain RLJCC1 and mother plant of Cymbopogon Commutatus is depicted in Table 1 which clearly indicated that strain RLJCC1 exhibited relatively better and consistent quality index stability values over the studied periods having high geraniol content, optimal range of geranyl acetate and very low citral content than those of C.commutatus mother plant. No marked differences in essential oil contents of mother plant and strain RLJCC1 were observed.~~

## Marked-Up Copy of Substitute Specification Filed July 9, 2003

Table 1

Essential oil quality index values and essential oil content of mother plant and  
selectant RLJCC1 (under rainfed)

Plant selectant Studied Period (year)	Percentage of				
	Geraniol	Geranyl acetate	Total of geraniol and geranyl acetate	Citral	Essential oil Content (%, w/w) on fresh weight basis
Mother plant (1994)	34.40	28.00	62.40	18.33	0.4-0.5
Selectant RRLJ CC1 (1995)	59.65	19.93	79.58	14.40	0.4-0.5
Selectant (1996)	62.16	18.08	80.24	12.75	0.4-0.5
Selectant (1997)	65.90	16.64	82.54	10.31	0.45-0.5
Selectant (1998)	67.37	15.22	82.59	6.35	0.45-0.5
1999RLJCC1	74.50	9.20	83.70	5.0	0.45-0.5

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

**PLANT ADAPTABILITY**

~~———— Cymbopogon Commutatus (RLJCC1) has high survival under adverse environmental conditions due to the presence of drought tolerance value, which is as high as 12% coupled with faster regeneration and early vigorous growth. Hence, it can withstand meteorological drought. The drought tolerance values of other Cymbopogon species having similar chemical composition are presented in Table 2.~~

**Table 2**

**Drought tolerance value of Cymbopogon species**

Plant species	Drought tolerance value (%)
Cymbopogon Commutatus RLJCC1	12
Cymbopogon Commutatus	10.5
Cymbopogon nardus var. confertiflorus RRL-CN-5	9

**VARIETY DESCRIPTION INFORMATION**

~~———— This invention is directed to methods for production of drought tolerant variety of C.commutatus by mass selection method under Jammu conditions and finally evaluated under field trials at sub-tropical belts/drought prone areas of District Kathua (Jammu & Kashmir State) as depicted in Table 3.~~

## Marked-Up Copy of Substitute Specification Filed July 9, 2003

**Table 3****Morpho-economic characters of drought tolerant variety of *C. commutatus***

Parameter(s)	Plant species		
	Values		
	RLJCCI	<i>C. commutatus</i>	RRL-CN-5
Plant height (to flowering tip)(cm)	104	109	115
Number of tillers plant <sup>-1</sup>	66	30	40
Rate of tillerization	2.0	1.8	1.9
Survival of plants (%)	85	75	80
Herbage plant (g)(Semidry)	175	170	200
Number of leaves	159	152	150
Leaf length (cm)	50	48	60
Leaf width (mm)	68	6.5	6.5
Essential oil content (% w/w)	0.45	0.40	0.45
Essential oil production hectare <sup>-1</sup> (litres)	78.8	75.0	80.0

Other plant characteristics are as under:

A. Quality indices

Geraniol (%) = 74.5

Geranyl acetate (%) = 9.2

Citral (%) = 3.0

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

**B. Plant Maturity**

~~Best adapted for drought prone areas of the sub-tropical belts.~~

~~Requirement of growing degree days for attainment of crop maturity in  
terms of essential oil quality indices at blooming & 6th leafing phenophase  
≈ 2900 (°C days)~~

**C. Color description**

~~Foliage green colors and sample reference No. 30D5.~~

**D. Odour evaluation of essential oil**

~~Grassy green note along with rosy note.~~

~~A spicy suggestion is also present.~~

**~~BRIEF DESCRIPTION OF THE ACCOMPANIED DIAGRAMS~~**

~~Fig. 1 shows the inflorescence-bearing shoot.~~

~~Fig. 2 shows pedicelled & sessile spikelets~~

~~(2.1 Palca, 2.2 Sessile spikelet, 2.3 Pedicellate spikelet)~~

~~Fig. 3 shows the adventitious roots~~

~~Fig. 4 shows mother plant of Cymbopogon Commutatus~~

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

- ~~—— Fig. 5 shows drought tolerant strain RLJCC1 (*C.commutatus*)~~
- ~~—— Fig. 6 shows well-grown plant of RLJCC1~~
- ~~—— Fig. 7 shows field view of RLJCC1~~
- ~~—— Fig. 8 shows the RAPD profile of *Cymbopogon Commutatus* (RLJCC1) and its mother plant *C.commutatus* (CCM) with primer 27 using optimum concentrations: primer 27 (48 ng) and MgCl<sub>2</sub> concentration of 2.5 mM. The RAPD profile of primer 27 in *C.commutatus* (RLJCC1) & its mother plant *C.commutatus* (CCM) is depicted in Lanes 3 & 4, respectively.~~
- ~~—— Fig. 9 shows the RAPD profile of *Cymbopogon Commutatus* (RLJCC1) and its mother plant *C.commutatus* (CCM) with primer 27 using optimum concentrations: primer 27 (48 ng) and MgCl<sub>2</sub> concentration of 2.5 mM. The RAPD profile of primer 27 in *C.commutatus* (RLJCC1) & its mother plant *C.commutatus* (CCM) is depicted in Lanes 3 & 4, respectively.~~
- ~~—— Fig. 10 shows the RAPD profile of *C.commutatus* (RLJCC1) and its chemically comparable plant species *C.nardus* var. *confertiflorus* RRL-CN5 with primers 27 and 29 using optimum concentrations of the primer 27 (48 ng) and MgCl<sub>2</sub> (2.5 mM) in both the plants species. In case of primer 29, the best profile was obtained using primer concentration of~~

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

~~33 ng and MgCl<sub>2</sub> concentration of 2.5 mM. The RAPD profile of primer 27 in *C. commutatus* (RLJCC1) & *C. nardus* var. *confertiflorus* (RRL-CN5) is depicted in Lanes 1&2 while that of primer 29 in *C. commutatus* (RLJCC1) and *C. nardus* var. *confertiflorus* (RRL-CN5) is depicted in Lanes 3&4, respectively.~~

~~Fig. 11 shows the RAPD profile of primer 22 in *C. commutatus* (RLJCC1) & its chemically comparable plant species *C. nardus* var. *confertiflorus* (RRL-CN5) using optimum primer concentration (33 ng) & MgCl<sub>2</sub> concentration (2.5 mM). The RAPD profile of primer 22 in *C. commutatus* (RLJCC1) & *C. nardus* var. *confertiflorus* (RRL-CN5) is depicted in Lanes 3&4. The exact PCR protocol followed is given as under:~~

**DETAILED DESCRIPTION OF THE NEW VARIETY**

The plants described were grown in the field at the Jammu and Kashmir State of North India. Slips were planted during February. Leafing and tillerization started during March with the rise in ambient temperature, and flowering began in April and continued to the 5<sup>th</sup> and 6<sup>th</sup> leafing stage during May and June.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

Color is specified by reference to the Methum Handbook of Colours by A. Kornerup and J.H. Wanscher, revised by Don Parey, Third Edition (1978), published by Erye Methuen, London, except where otherwise indicated.

The asexual reproduction achieved by the planting of slips and the rooting of cuttings has confirmed that the combination of characteristics of the new 'RLJCC1' variety are firmly fixed. Such characteristics are transmitted true to type to subsequent generations.

The new variety of the present invention can be readily distinguished from its mother plant of the *Cymbopogon commutatus* species. More specifically, the 'RLJCC1' variety displays lighter green leaves, is more drought tolerant and inherently produces a more advantageous essential oil having an enhanced geraniol and geranyl acetate content.

The new variety when fully mature commonly achieves a maximum height of approximately 100 to 150 cm. The canopy spread of the plant commonly is approximately 50 cm, and the basal area of a mature plant commonly is approximately 960 cm<sup>2</sup>.

The upper surface of the leaf blade is 30D5 in coloration. This can be compared to a coloration of 27E5 typically displayed by the mother plant of *Cymbopogon commutatus*. Stated differently the leaves of the species commonly



**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

are dull jade green as shown in FIGURE 5, while the leaves of the 'RLJCC1' variety are a lighter green as shown in FIGURE 4. The coloration of under surface of the leaves of 'RLJCC1' is 27E5. The leaf margin is entire and leaf surface is smooth on the upper surface and somewhat rough on the under surface. The leaf blade is flat and linear and commonly measures approximately 50 cm in length. The leaf blade width commonly is approximately 5 to 8 mm at the widest point. The leaf apex is filiform.

\_\_\_\_\_ The culms commonly are approximately 30 to 50 cm in length and approximately 4 to 5 mm in width. Their texture is smooth and the coloration is Timber Green, 6H, Plate 18 by reference to "A Dictionary of Color" by A. Maerzard and M. Reapaul, Second Edition (1950) by McGraw Hill Book Company of New York.

\_\_\_\_\_ An extensive highly branched rooting system is formed as illustrated in FIGURE 3. The primary root is divided into many fibrous branches that commonly display a coloration of light brown, 1A, Plate 18 by reference to "A Dictionary of Color". A root length of approximately 10 to 15 cm commonly is observed.

\_\_\_\_\_ Commonly 10 to 12 nodes are present with 1 to 3 lateral branches at each node.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

The ligules are scarious, rotundate, and measure approximately 4 to 5 mm.

The inflorescence commonly appears in March-April and September-October. A synchronous mass flowering pattern is observed. A spatheate panicle of paired divariate racemes is formed having a length of approximately 50 to 100 cm. The color is light brown, 1E18.

The spikelets commonly are 4 to 5 mm in length, sessile, are linear-lanceolate, acuminate, as well as pedicelled in pairs. The pedicels are villous and the spathes are approximately 1.5 to 3 cm in length.

The glume is boat-shaped, pilose on the back, the base margin is ciliate, the lower lemma hyaline linear-lanceolate, and the upper lemma is hyaline. The awn is slender and geniculate, and there are two lodicules.

The anther is ditheous, extrose and versatile. The stigma is unilocular and superior globose with one basal ovule and two feathery stigmas elevated on two separate styles.

The seeds are oblong, plano-convex in configuration, and brown in coloration, Plate 7, Row 10 of "A Dictionary of Color".

During observations to date the pest and disease resistance/susceptibility is believed to be comparable to that of the species.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

The quantity of essential oil produced by the new 'RLJCC1' variety is believed to be generally comparable to that of the species. However, the content of the essential oil is considered to be superior to that of the species with more geraniol and geranyl acetate being present. Also, the citral content of the essential oil was lower than that commonly displayed by the species.

In the following Table 1 the essential oil content of the new 'RLJCC1' variety and the typical mother plant of *Cymbopogon commutatus* are compared.

**Table 1**

<u>Plant</u>	<u>Percentage of</u>				<u>Essential oil Content (% w/w) on fresh weight basis</u>
	<u>Geraniol</u>	<u>Geranyl acetate</u>	<u>Total of geraniol and geranyl acetate</u>	<u>Citral</u>	
<u>Mother plant</u>	<u>34.40</u>	<u>28.00</u>	<u>62.40</u>	<u>18.33</u>	<u>0.4-0.5</u>
<u>'RLJCC1'</u>	<u>74.50</u>	<u>9.20</u>	<u>83.70</u>	<u>5.0</u>	<u>0.45-0.5</u>

It will be noted that the combined geraniol and geranyl acetate content of the essential oil of the 'RLJCC1' variety exceeds 80 percent by weight. The citral content in this instance was only 3.0 percent by weight.

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

The drought tolerance performance of the new 'RLJCC1' variety has been found to be superior to that commonly exhibited by the species and by the related *Cymbopogon nardus*, var. 'RRL-CN5'. Drought tolerance observations are summarized in the following Table 2.

**Table 2**

<u>Plant</u>	<u>Drought Tolerance Value (%)</u>
<u><i>Cymbopogon Commutatus</i> 'RLJCC1'</u>	<u>12</u>
<u><i>Cymbopogon Commutatus</i></u>	<u>10.5</u>
<u><i>Cymbopogon nardus</i> var. 'RRL-CN-5'</u>	<u>9</u>

When the new 'RLJCC1' variety was grown in a large scale field trial and compared to typical *Cymbopogon commutatus* and to the 'RRL-CN5' variety, the comparative data presented in Table 3 was observed.

## Marked-Up Copy of Substitute Specification Filed July 9, 2003

Table 3

Parameter	Plant		
	'RLJCCI'	<i>C. commutatus</i>	'RRL-CN5'
Plant height (to flowering tip)(cm)	104	109	115
Number of tillers per plant	66	30	40
Rate of tillerization	2.0	1.8	1.9
Survival of plants (%)	85	75	80
Herbage/plant (g)(Semi-Dry)	175	170	200
Number of leaves	159	152	150
Leaf length (cm)	50	48	60
Leaf width (mm)	68	65	65
Essential oil content (% w/w)	0.45	0.40	0.45
Essential oil production in liters per hectare	78.8	75.0	80.0

The RAPD profiles of the selected hybrid (RLJCC1) 'RLJCC1' variety, its *Cymbopogon commutatus* mother plant (CCM) and the plant related to it chemically (RRL-CN5) , and the 'RRL-CN5' variety using the above-mentioned primers (Table 4) are very specific and identified in Table 4 can be used for the identification of to distinguish the specific cultivars/chemotypes.

Table 4

## Name and sequence of primers used

Primer-code	Operon Random primer	Primer sequence
22	OPA02	TGCCGAGCTG
27	OPA07	GAAACGGGTG
29	OPA09	GGGTAACGCC

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

**Table 4**

<u>Primer code</u>	<u>Operon Random Primer</u>	<u>Primer Sequence</u>	<u>Sequence I.D.</u>
<u>22</u>	<u>OPA02</u>	<u>5'-TGCCGAGCTG-3'</u>	<u>1</u>
<u>27</u>	<u>OPA07</u>	<u>5'-GAAACGGGTG-3'</u>	<u>2</u>
<u>29</u>	<u>OPA09</u>	<u>5'-GGGTAACGCC-3'</u>	<u>3</u>

See in this regard the results reported in FIGURES 8 to 11.

**The PCR protocol for this determination is set forth below:**

92-95°C-	2-4 min x 1 cycle <sub>1</sub>	
92-95°C-	1-2 min	} x 30-45 cycles <sub>1</sub>
32-38°C-	1-2 min	
72°C-	1.5-2.5 min	
72°C-	4-7 min x 1 cycle <sub>1</sub>	

The reaction assay mixture was prepared as ~~under~~ set forth below:

DNA	-	7-20 ng <sub>1</sub>
10 x buffer	-	2-3 ml <sub>1</sub>
<del>MgCl<sub>2</sub></del> <u>MgCl<sub>2</sub></u>	-	1.5-2.5 mM <sub>1</sub>
dNTP mix	-	150-250 mM <sub>1</sub>
H <sub>2</sub> O <u>H<sub>2</sub>O</u>	-	As per requirement <sub>1</sub>
Primer	-	20-40 ng <sub>1</sub>

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

Taq. Pol. Enz. - 0.5-2.0 U<sub>1</sub>  
20-30 µl reaction<sub>1</sub>

~~Fig. 12 shows mother plant of C.commutatus (CCM) at vegetative stage.~~

~~Fig. 13 shows droughts tolerant strain RLJCC1 at vegetative stage.~~

~~(The figures 12 & 13 are for foliage color descriptions).~~

**ADVANTAGES**

- ~~1. High regeneration vigor of the tillers.~~
- ~~2. More than 85% survival of tillers and faster growth capacity and quick leaf induction. Hence, it would be an ideal variety for commercial cultivation especially under drought prone areas/belts of the Indian regions.~~
- ~~3. C.commutatus (RLJCC1) essential oil can be used extensively for imparting rosaceous aroma to wide range of products in perfumery, soaps & flavor and food industry. Apart from the occurrence of geraniol (74.5%) and geranyl acetate (9.20%) in its essential oil, a high value product ocimene (5-7%) is also present, which is extensively used in high-grade perfumes.~~

**Marked-Up Copy of Substitute Specification Filed July 9, 2003**

- ~~4. The essential oil is devoid of unusual menthadienols, as reported in Sudanese *C. commutatus* essential oil upto 87%. Banthorpe, D.V., Duprey, R.J.H., Hassan, M., Janes, J.F. and Modawi, B.M. 1976. Planta Medica 29:10-19.~~
- ~~5. This oil also provides protection against mosquito bite i.e., acts as a mosquito repellent.~~

The essential oil of the 'RLJCC1' variety has been found to be substantially devoid menthadienols which have previously been reported in the essential oil of *Cymbopogon commutatus*. Also, the essential oil of the new variety has been found to repel mosquitos.

The new 'RLJCC1' variety has not been observed to date under all possible environmental conditions. Thus, it is possible that the phenotype may vary somewhat under different environmental conditions.